Enkele aspecten van competentie bij Bacillus subtilis

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SUMMARY

The results reported in this thesis refer to: (1) an analysis of the variation in transformability of *Bacillus subtilis* cultures when grown to competence both by the same method and by different methods, (2) the estimation of the size of the competent fraction by two methods and (3) an electron microscopic and autoradiographic investigation of submicroscopic aspects of competence and of the intracellular transport of transforming DNA.

Substantial variation in transformability may exist in cultures grown to competence by the same method. This variation is mainly due to variation in the size of the competent fraction in the cultures. The amount of DNA absorbed per competent cell, the efficiency of competent cells to integrate the absorbed DNA and the extent of phenotypic expression appear to be subject to only slight variation. The transformability of a culture during development of competence and the size of the competent fraction increase proportionally, whereas the amount of DNA absorbed per competent cell remains fairly constant, so that also during development of competence the increase in transformability is practically exclusively attributable to an increase in the size of the competent fraction (chapters III and IV).

When cultures are grown to competence in a medium in which the casein hydrolysate is replaced by competence enhancing amino acids (Wilson and Bott, 1968), the increased transformability, which is observed, appears to be caused by an increase in the size of the competent fraction as well as by a larger amount of DNA absorbed per competent cell. The efficiency of integration of donor markers and of their phenotypic expression is not affected by the change in the composition of the medium in which the cells are grown to competence (chapter IV).

The size of the competent fraction in a *ade trpC2* culture, after transformation with wildtype DNA, determined by Goodgal and Herriott's method (1961) from the frequencies of ade^+, $trpC2^+$ and ade^+ $trpC2^+$ transformants (percentage competent cells $= \frac{\%\ ade^+\ \text{transformants} \times \%\ trpC2^+\ \text{transformants}}{\%\ ade^+\ trpC2^+\ \text{transformants}}$), appears to agree reasonably well with the fraction of labelled cells determined by light microscopic autoradiography after transformation with ^{3}H-thymidine labelled DNA (chapters III and IV). However, it was expected that the size of the competent fraction determined by Goodgal and Herriott's method would be overestimated by at least a factor two. As a consequence of random integration of one of the strands of a DNA duplex – only one of the strands of a transforming DNA molecule is integrated – a cell having absorbed two unlinked markers will have a 50% pro-

bability of giving rise to a colony in which both markers are in the same cell, provided that the DNA is integrated in one nucleoid only. However, integration of DNA is in all probability not restricted to one nucleoid only. In chapter VI evidence is presented that 50% of the competent cell fraction exists of cells with two nucleoids and that in a fraction of these cells transforming DNA can be integrated in both nucleoids. Using Goodgal and Herriott's method, this results in an overestimation of the size of the competent fraction by a factor of maximal 2,67, in stead of by a factor 2. The overestimation will be reduced however, if correction of heteroduplex DNA and integration of a second *ade*$^+$ and/or *trpC2*$^+$ marker occurs (chapter III).

From a clonal analysis of *ade trpC2* cells, transformed for both an *ade*$^+$ and a *trpC2*$^+$ marker, it appears that the number of cells in which the genetic information of both markers is incorporated into the same recipient DNA strand exceeds considerably the number of cells in which this information is incorporated in different strands. The colonies formed vary considerably with respect to their genotypic composition. The various colony types may be explained both by integration of a second *ade*$^+$ and/or *trpC2*$^+$ marker and by correction of heteroduplex DNA. From the frequencies of the various colony types it is possible to deduce that in all probability correction of heteroduplex DNA has occurred (chapter V).

Also in chapter V results are reported which show that the size of the competent fraction determined by Goodgal and Herriott's method depends upon the combination of markers used. Especially those combinations in which the *trpC2*$^+$ marker participates yield the lowest value for the size determined. The most plausible explanation for this phenomenon is that the extend of correction of heteroduplex DNA is dependent upon the nature of the marker which forms part of the heteroduplex.

Electron microscopic examination (chapter VI) shows that both the frequency of cells with mesosomal nucleoid-plasmamembrane connections and the number of these connections per cell increase during competence development in a culture. Moreover, it appears from electron microscopic autoradiography that competent cells have more mesosomal nucleoid-plasmamembrane connections than non-competent cells. Directly after uptake, the transforming DNA is found to be associated with mesosomes in tip, middle and cross-wall zones (see figure VI.5 on page 86). The DNA does not seem to associated with mesosomes in intermediate zones. In the tipzones almost exclusively mesosomes were observed which are connected with the plasmamembrane only. After uptake, the DNA migrates as a function of time towards mamembrane only. After uptake, the DNA migrates as a function of time towards the nucleoids. During this transport the DNA seems to remain associated with mesosomes, presumably with the mesosomal nucleoid-plasmamembrane connections. The absorbed DNA migrates in all probability from the peripheral middle zones towards the nucleoids, on the understanding that the DNA absorbed in the tip-

zones initially is transported in a peripheral zone, probably the periplasmic space or its boundary, towards the peripheral middle zones in which, in contrast with the tipzones, mesosomal nucleoid-plasmamembrane connections do occur. During the period from 15 to 60 minutes after termination of uptake, the DNA becomes fully associated with the nucleoids.

In addition to a larger number of mesosomes, the competent fraction also differs from the non-competent fraction by a higher degree of variation in the length of the cells and a higher frequency of mesosomal nucleoid-plasmamembrane connections in the middle zones compared to the frequency in the cross-wall zones.

Approximately 50% of the competent cells has one nucleoid, the remainder possesses two; in at least a fraction of the binuclear competent cells DNA incorporation seems to occur in both nucleoids.